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EFFECT OF IDENTICAL AND ALTERNATING LIGHT-DARK PERIODS ON THE GROWTH, DRY-MATTER ACCUMULATION AND CARBOHYDRATE CONTENT OF MAIZE LEAVES

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Abstract

The growth of Pioneer 3839 hybrid maize leaves, the changes in the amount of dry-matter and soluble sugar and starch levels of the leaves were studied for 37 days at identical light-dark periods (LDP) of 16–8 hours, 30–15 min. and 15–7.5 min.; as well as on plants grown in changed LDP-s following the first 22 days of growth. The daily period of light was 16 hours, the light intensity was 32 W/m², and the temperature was 20±2 °C.

It could be determined that there was slight increase in the dry-matter mass of the plants growing for 37 days at identical LDP-s on the effect of the 30–15 min. LD-treatment, and a decrease was observable on the effect of the 15–7.5 min. LDP, compared to the 16–8 hours control.

Among the maize plants grown in alternating LDP-s in every case the dry-weight of ones grown in shorter LDP-s (30–15 min. 15–7.5 min.) for the first 22 days, and than were exposed to 16–8 h LDP for 16 days, — was higher.

The short periodical illumination accelerates the development and longitudinal growth of the young leaves in general. The changes in the LDP-s do not have any effect on the growth rate of the half-developed leaves.

In the developed leaves grown in 30–15 and 15–7.5 min. LDP-s for 37 days the decrese of the soluble sugar content is considerable, and the starch-content shows a decrease of slighter degree. In the 30–15 min. LDP the soluble carbohydrate level of the young leaves (6th, 7th) only slightly varies; the starch amount does not change at all.

From the changed LDP-s applied after 22 days of growth the shorter ones decrease, while the longer ones increase the amount of soluble sugar and starch.

Key-words: light-dark period, dry-weight, soluble sugar, starch, growing, maize.

Introduction

The alternating short light-dark periods (LPD) frequently decrease the relative efficiency of photosynthesis; respectively, it comes close to, but only rarely surpasses the values measured in continuous light (SAGER and GIGER, 1980). This effect is particularly striking in the case of algae, in respect to plants of higher order it appears less unambigously.

From the latest results, on the basis of the studies carries out by NAYLOR and GILES (1982) on bean plants, the 5–5 min. and 1–1 min. LDP-s decreased the dry-weight and leaf surface, and increased the quota of the root. These also decreased the amount and size of starch granules in chloroplasts, and furthermore, had significant on the pigment formation.

There are still relatively few data, regarding the effect of the varying LDP-s on the carbohydrate metabolism. It seems that our knowledge concerning the regu-

lation of the biosynthesis and decomposition of strach is not sufficient enough yet for explaining acceptably the starch level-decreasing effect of the short light-dark periods (PREISS, 1982).

According to the studies of KAISER and BASSHAM (1979) — following a single short, 4 min. dark period the CO_2 fixation and the starch synthesis continues in a slightly decreased degree, although without lag phase.

According to our studies so far (MARÓTI and PATAKY, 1982; MARÓTI and MIHALIK, 1982; TAKÁCS and MARÓTI, 1983; MARÓTI and TAKÁCS, 1983), besides continuous light intensity and identical daily light amount the effect of alternating illumination is determined by the length of the light-dark periods and by the genotype of the maize. On the basis of the changes in dry matter production the P523 maize type is capable of accomodating to the 30–15 and 15–7.5 min. LDP-s the best, while this ability is the least in the case of the P165 maize type.

In the case of the P523 maize, compared to the long-day (16–8 hours) illumination, the 30–15 min. LDP accelerates the development of the leaves increases the leaf surface and the dry weight per unit surface (MARÓTI and MIHALIK, 1982).

In respect to the plants studied so far, the uniform unfavourable effect of the 15–7.5 min. LDP was mainly manifested in the decrease of specific leaf-dry weight, the relative increase of root-weight, and in the significant decrease of chlorophylls and carotenoids (MARÓTI, 1982).

Compared to the parent lines, the P3901 hybrid maize made the best use of the light in every LDP, and its considerable mass of dry matter was accompanied by the high soluble sugar concent of the leaves (MARÓTI and MIHALIK, 1982).

The certain maize types are adaptable to the short LDP depending on their genotype. It is also the characteristic effect of the 30–15 min. LDP that the soluble sugar and starch amount found in the unit dry matter of the leaf decreases depending on the genotype. However the 15–7.5 min. LDP is uniformly and expressedly unfavourable for the accumulation of carbohydrates (MARÓTI and MIHALIK, 1982). The question is, how the soluble sugar and starch change in the leaves of maize adapted to varing LDP-s, and then exposed to a new, shorter or longer LDP? Those leaves are of particular interest from this viewpoint, which grew partly (half time) in the earlier, partly in the new LDP; and respectively, those leaves, which were formed entirely in the new LDP, i. e., the observable initiative leaves appeared after the transfer.

Answering the above question, this paper reports on and discusses the changes in dry matter, soluble sugar and starch levels of Pioneer 3839 type hybrid maize, developing on the effect of identical and alternating LDP-s.

Materials and Methods

Pioneer 3839 hybrid maize (*Zea mays* L.) were grown in phytotron (HORVÁTH, 1972) for the experiments. The temperature was 20 ± 2 °C, the humidity of the air was 50–70%.

The plants were sown in the mixture of sand-perlite in the ratio of 1:1, the nutriment was ensured with modified Hoagland nutrient solution, and the moisture-content corresponding to 80% of water capacity was maintained daily by tap-water with watering according to weight. Two series of experiments were carried out at varying time-points.

F-29 light tubes were applied for illumination (wave-lengths 400–700 nm). The light flux density was 32 W/m². The total illumination of 16 hours daily was continuous in the first climate chamber,

lasting from 8 a.m. to 12 p.m. (16–8 hours light-dark period), in the second chamber alternating periods of 30 min. light and 15 min. dark were applied: 30–15 min. LDP; and in the third, 15 min. light and 7.5 min. dark periods alternated: 15–7.5 min. LDP. The daily total illumination was 16 hours in every chamber.

After growing for 22 days the plants were divided into 3 equal parts in all three climate chambers: 12 plants remained in the original place, the rest of the plants were placed into new LDP-s; 12 into one, 12 into the other. 3–3 plants were selected from each variant and these were followed by daily measuring of the growth in length of their 4th and 5th leaves, counted from the bottom.

The plants were processed at the age of 37 days. The fresh weights of the 12–12 plants per treatment were measured according to organs. This processing was always carried out in the morning hours. The dry weight was measured after drying at 70 °C.

The carbohydrate content was determined from the 4th and from the youngest 6th, 7th... leaves. The dry matter of the appropriate leaf from 7 plants per treatment was combined and cut into entirely small pieces with scissors. From this, 50 mg were ground with some water with the help of quartzsand.

The soluble carbohydrates were extracted with 10 ml hot water, then following centrifugation and discharging, extraction was performed again with newer 10 ml hot water by shaking for 15 min. The two supernatants were blended, filled up to 100 ml, from which the determinations were accomplished.

After extracting the soluble sugars a quantity of 10 ml 35% perchloride acid was poured onto the centrifugal sediment. Following 1 hour of shaking this was centrifuged, then after pouring off the supernatant, a newer amount of 10 ml 35% perchloride acid was added and after 1 hour's shaking the samples were left till the following day. Next day the mixture was centrifuged and the supernatant was combined with the first perchloride acidic fraction. After appropriate dilution, the starch content was determined.

The determination of the soluble sugars and the hydrolysed starch was performed using the method of DUBOIS et al., (1956): 0.5 ml sample + 0.5 ml 5% phenol + 2 ml concentrated sulphuric acid. After 20 minutes the cooled coloured solution was measured by photometry at 485 nm. The calibration curve was prepared with D-glucose.

In the case of each sample the carbohydrate content was determined twice from 50 mg dry matter independently of each other, and the measurings were repeated when they were necessary.

Results

A) The effect of identical and alternating short LDP-s on the accumulation of the dry-matter and percental quota of dryweight of organs in 3839 maize.

It can be seen from the measurings of the dry-weight related to one plant (Table 1) that the mass of dry-matter of the plants grown in same LDP for 37 days was slightly increased by the 30–15 min. LD-treatment and decreased by the 15–7.5 min. LDP compared to the 16–8 hours control. The uniform effect of the short periodical illumination (30–15 and 15–7.5 min) was that it stimulated the development and increase in dry-weight of the young leaves (Figs. 2, 3), and decreased the amount of dry-weight in the developed leaves (Fig. 1).

The 30–15 min. LDP being the same throughout the experiment firstly increased the dry-matter percental quota of the roots (Table 1).

In the case of alternating LDP-s, at the timepoint of transfer to new LDP, the 4th leaf was cc. half developed (the leaf was at about half-time between appearance and complete development), the 5th leaf just appeared and the 6th, 7th leaves appeared in the new LDP during the 16 days.

From the maize grown in alternating LDP-s in every case the dry weight of those which grew in shorter LDP (30–15 and 15–7.5 min) for the first 22 days, and then were transfered to 16–8 hours LD periods for 15 days was higher (Table 1).

A smaller degree of increase in dry-matter compared to the continuous 16–8 hours control was observable even when the plants were placed from longer (16–8 hours and 30–15 min.) LD periods after 22 days to shorter (15–7.5 min) ones.

In the alternating LD periods the accumulation in dry-matter was generally in relationship with the percental increase of the root and leaf sheath.

Table 1. The effect of LDP-s being the same throughout the experiments and changed after 22 days of growing, resp., on the dry-weight (mg/plant) and quota of organs of 37 days old Pioner 3839 maize.

In the 16-8 hours, 30-15 min. and 15-7.5 min. LDP-s the 36-36 identical plants were grown for 22 days, then divided into three according to treatment : 12 plants remained in the originai place, and the rest of the 12-12 plants were grown further in other two LDP-s.

The daily amount of light (512 Wh/m²) was the same in each variant. The plants were processed at the age of 37 days. The data are the averages of the 12-12 plants from the two experimental series.

Light-dark period		Dry-weight (mg/plant)							
of the first 22 days	on the further 15 days	root		leaf sheath + stem		leaf		total	
		mg	%	mg	%	mg	%	mg	%
16-8 hours	16–8 hours	383	39	167	18	415	43	965	100
	30–15 min	383	39	175	18	412	43	970	100
	15–7.5 min	450	40	279	25	388	35	1117	100
30–15 min	16–8 hours	538	45	183	15	379	40	1200	100
	30–15 min	421	43	142	14	418	42	981	100
	15–7.5 min	517	45	159	14	461	41	1137	100
15–7.5 min	16–8 hours	513	43	204	17	478	40	1194	100
	30–15 min	374	39	150	16	426	45	950	100
	15–7.5 min	367	40	150	16	406	44	923	100

The dry-weight of the 4th leaf was strongly decreased by the transfer to shorter LDP, however, that of the same leaf of plants placed from shorter LD period to longer one increased to such extent that it even surpassed the data gained for plants grown throughout in 16–8 hours LDP (Fig. 1). That is, the leaf which obtained advantage in development in shorter LDP, grew for longer period when transfered to longer LDP, therefore its dry-weight became higher than that of the leaf placed with disadvantage in development from longer LDP to shorter LDP. This effect was particularly striking in the case of combinung the 16–8 hours and 15–7.5 min. LDP-s (Fig. 1), but the same effect could be recognized in slighter form in the case of the alternated 16–8 hours and 30–15 min. LDP-s, and even in a less distinct form on the occasion of changing the 30–15 min. and 15–7,5 min. LDP-s, resp. (Fig. 1).

The dry-weight of the young 6th, 7th leaves developing in new LDP after 22 days was regulated by two effects. On the one hand, the short periodical illumination stimulated the development of the young leaves; on the other hand, the maize placed from pre-

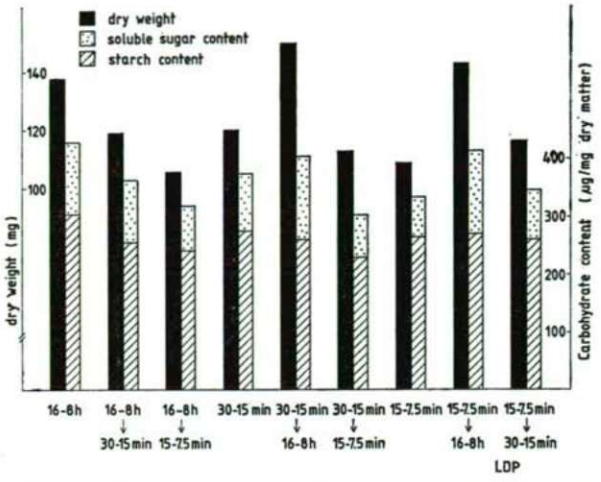

Fig. 1. The carbohydrate content and dryweight of the 4th leaf on the effect of identical and alternating LDP-s.
Experimental conditions: see Table 1.
At the time of transfer to the new LDP the 4th leaf was about half-developed.
The determination of the carbohydrate content was accomplished from the combined and homogenized dry-matter of the 4th leaves from 7 plants per treatment, measuring 50 mg twice.
The data are the averages of 2 experimental series.

vious short LDP to longer LDP produced more dry-matter than those which grew throughout in either short or long LDP. This is why the dry weight of the young 6th, 7th leaves was found to be the least in the case of maize grown entirely in 16–8 hours LDP, from every experimental varient (Fig. 2).

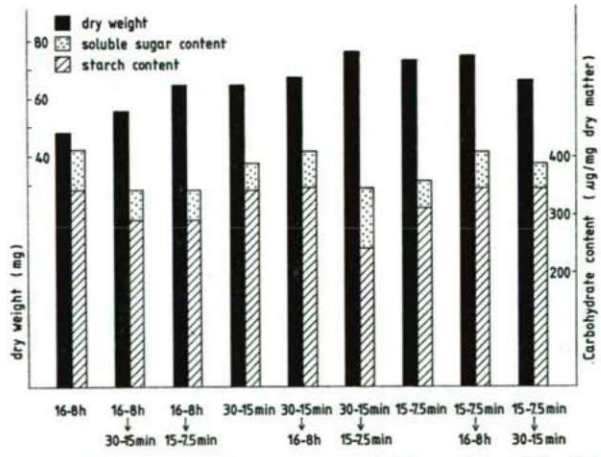

Fig. 2. The development of the carbohydrate content and dry-weight of the 6th, 7th leaves on the effect of identical and alternating LDP-s. For experimental conditions and studying methods see Table 1 and Fig. 1.
The 6th leaf had not appeared yet at the time of transfer to new LDP.

B) The effect of identical and alternating LDP-s on the longitudinal growth of the 4th and 5th leaves of the maize .

The length of the 4th and 5th leaves was measured daily after placing the plants into new LDP (22 days). The end of the 3rd leaf sheath was taken as the lower source of the leaf length. At the beginning of the measurings the 4th leaf was in half-time of development and the 5th leaf had just appeared. On one part, it could be observed from the measurings that the development of the leaves was accelerated by the short light-dark periods and the leaves reached complete development earlier. On the other part it was observable that the effect of the various LDP-s greatly depended on the age and state of development of the leaves.

The new LDP hardly showed effect on the growth of the half-developed 4th leaf, and it grew at the rate characteristic to the original LDP even after transfer (Fig. 3).

At the time of change in LDP, the 5th leaf had only started to grow, and it rapidly became adapted to the growth rate characteristic to the new LDP. It can also well be seen that at the beginning the plant grew more rapidly in the 15–7.5 min LDP (Fig. 3).

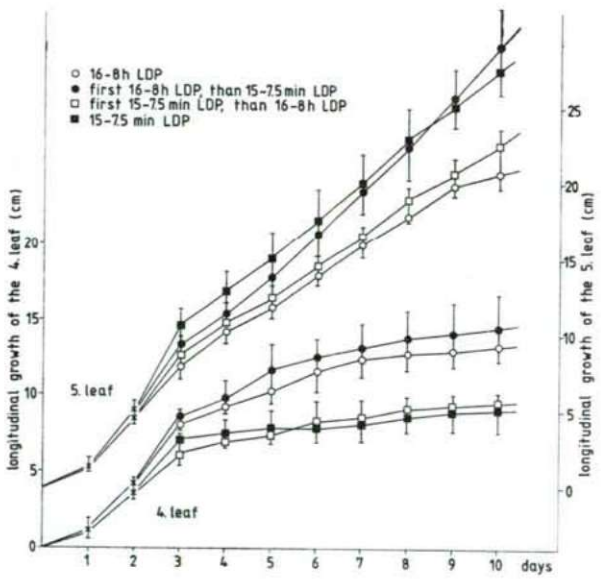

Fig. 3. The effect of identical and alternating LDP-s on the longitudinal growth of the 4th and 5th leaves.
The measurings were commenced when the plants were 22 days old, at the time of their placing into new LDP. The source of the length-measurings was all throughout started from the lower edge of the 3rd leaf's lamella since at this time-point the lingule and auricle of the undeveloped 4th leaf had not been formed yet, the leaf was in cc. half-time state of growth, and the 5th leaf had only just appeared.
The measurings were taken for a period of 10 days from 3–3 plants daily.

C) The effect of identical and alternating LDP-s on the carbohydrate content of the developed (4th) and developing (6th, 7th) leaves.

The shorter light-dark period decreased the level of soluble sugar and starch in the case of the studied leaves; that of the soluble sugar at higher degree, and that of starch at lower degree (Figs. 1, 2). Related to dry-weight unit, the soluble carbohydrate content of the 4th leaf showed 33% decrease in the 30–15 min. LDP compared to the 16–8 hours LDP, and a decrease of 53% was experienced in the 15–7.5 min LDP (Fig. 1). The change in starch level was slighter: of 10–20%.

The carbohydrate level of the developing leaves (6th, 7th) showed lower decrease in the case of 30–15 min. and 15–7.5 min. LDP. In the 30—15 min. LDP there was no, or only slight change in the level of starch (Fig. 2).

In the developing leaves in every case the starch level was found to be higher, and that of soluble sugar was lower, than in the developed leaves. It is presumable that the plant "ensures" higher starch level for the intensive growth of the young leaves nevertheless, the energy-consumption implying intensive growth keeps the soluble sugars at low level, i. e. there is no "stagnant" stock of soluble sugar.

This assertion is in agreement with the results of KAISER and BASSHAM (1979), as well as PREISS (1982), according to which the starch found in the leaf can easily be mobilized and is controlled by the effective regulation of the metabolic processes related to the various energy productions and consumptions.

The decrease in starch level developing in the shorter cycles is in good agreement with the results of TAKÁCS and MARÓTI (1983), and NAYLOR and GILES (1982), who found fewer amount of starch granules in chloroplasts in the case of cycle lengths between 30 and 5 min., than in chloroplasts of plants grown under continuous light.

From the new LDP-s to which the plants were transfered at the age of 22 days, the shorter decreased, and the longer increased the carbohydrate level; both in the case of starch and soluble sugars. That is, the second LDP (from the age of 22 to 37 days) had determinative effect on the carbohydrate level measured at the age of 37 days. Thus, for example, the carbohydrate level of the leaves placed from the 16–8 hours LDP to 15–7.5 min. LDP was found to be close to equal to the leaves receiving 15–7.5 min. LDP treatment throughout the experiment, and the carbohydrate level of the 4th leaf of the plants transfered from 15–7.5 min. LDP to 16–8 hours LDP also came close — although not reaching — that of the equivalent leaves exposed throughout to 16–8 hours LDP-s. It seems that the short periodical illumination caused a disturbance in the carbohydrate metabolism and even the plants placed to longer LDP at 22 days of age were incapable of complete "regeneration".

The developing leaves were also characterized by the above mentioned alterations, only in a slighter degree.

The determination in regard to the evaluation of the dryweight is also valid for the carbohydrate level, according to which the observed alterations were the most striking in the case of the combination of the 16–8 hours and 15–7.5 min. LDP-s, being similar but of decreased degree in the case of the other two combinations.

Discussion

The cellular biochemical and fine structural interpretation of the differing effects of alternating LDP-s is still scant (NAYLOR and GILES, 1982). Several theories are known: "photosynthetic saturation" (EMERSON and ARNOLDS, 1932), "endogeneous daily rhythm" (BÜNNING, 1935, 1950), "phytochrome control" (KIGEL and KOLLER, 1970; SCHOPFER, 1972; HURD, 1973), but in our opinion the more profound cause is unknown as yet regarding the effect of alternating LD periods.

It has been demonstrated (MARÓTI and PATAKY, 1982; MARÓTI and MIHALIK, 1982) that the dry matter-content increasing and decreasing effect, resp. of alternating LD periods (besides constant light intensity and identical daily amount of illumination) is determined by the length of the light, dark periods and by the genotype.

According to SAGER and GIGER (1980) the photosynthetic efficiency of the alternating short light-dark periods only rarely surpasses the value measured in continuous light. The rarity is disputable since several experiments are known in which the plants produce more dry-matter in the shorter LDP-s than in continuous light (GARNER and ALLARD, 1931; EMERSON and ARNOLD, 1932; BONDE, 1955; POLLARD, 1970; HORVÁTH and MIHALIK, 1977; MARÓTI and PATAKY, 1982, and others).

It is very likely that under cell level the alternating LDP-s firstly have effect in the chloroplast. In the short LDP-s the chloroplasts either swell or shrink, depending on the "genotype" and the length of the periods. The change in the volume of maize chloroplasts is in negative correlation with the dry-matter production in mesophyll cells, and in positive correlation in bundle sheath cells (MARÓTI and PATAKY, 1982). Concerning the structure of the chloroplasts, in the case of beans the characteristic effect of the 30–15, and 15–7.5 min. LDP-s is expressed by the almost complete lack of starch from the chloroplasts, the decrease in the thylakoid number per grana, the significant increase per unit surface of the stroma lamellae and end membrane length (TAKÁCS and MARÓTI, 1983).

The currently performed electronmicroscopic studies on the P 3839 maize (PATAKY, personal communication) also show that the organization of the inner membrane of chloroplasts is responsible for the fact that the maize grown earlier in short (30–15, and 15–7.5 min.) LDP make better use of the light in 16–8 hours LDP than the one, kept throughout in short or longer LDP-s.

According to our assumptions the varying effect of the short LDP-s at such a weak light intensity (32 W/m^2) is not determined by the dark, but firstly by the light reactions, and is in direct relationship with the speed of the proton-accumulation in the loculus of the thylakoid, as well as with the development in time of the electron stream of inductive phase (MARÓTI, 1982).

The effect of the alternating LDP-s above cell level may firstly be in relationship with the structure, size, carbohydrate content of the leaves and the trend and degree of the translocation. The size of the leaf area is in tight correlation with the carbohydrate content of the leaves (BOOYSEN and NELSON, 1975).

In our experiments, too the prominent dry-matter amount was accompanied by high soluble sugar and starch content in the LDP-s having favourable effect on growth.

According to the studies of NAYLOR and GILES (1982) carried out on beans the decrease in dry-weight is joined by the increase in the quota of roots in the case of

short LDP-s. This was not experienced by us in the 15–7.5 min. LDP, at the same time the placing of the plants from short LDP to longer LDP presented such an increase in the dry-matter quota of the roots which had only slight decreasing effect on that of the leaves.

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